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Potential of Denaturing Gradient Gel Electrophoresis for Scanning of β-Thalassemia Mutations in India

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Over the last few years, substantial progress has been made in developing strategies for the detection and characterization of various mutations causing β-thalassemia. The Indian population comprises of numerous endogamous caste groups and β-thalassemia is seen in almost all of them. Knowledge of the spectrum of β-thalassemia mutations in the population is a prerequisite for successful implementation of a prevention programme. Among the different approaches available today, Denaturing Gradient Gel Electrophoresis (DGGE) offers a valid technical approach which is applicable for screening of known mutants and polymorphisms as well as in locating regions of DNA bearing unknown mutations.

We analysed 356 unrelated β-thalassemia heterozygotes by DGGE and detected 30 anomalous DGGE patterns. Fifteen mutations were characterized after sequencing 25 anomalous patterns. Of these, codon 10(GCC → GCA) is a recently reported novel β-thalassemia mutation while −28(A → G) and codon 121(G → T) are being reported for the first time in the Indian population. HbS and HbE also showed two anomalous DGGE patterns each. Framework (FW) linkage studies showed that four mutations were associated with different β-globin gene frameworks. Linkage of IVSI-5(G → C) and cap site +1 (A → C) to FW2 and 619-bp deletion to FW1 is being observed for the first time.

Multiple DGGE patterns corresponding to the same mutation is one of the major drawbacks of this technique. In spite of this, if sufficient preliminary work has been carried out to compile a comprehensive catalogue of DGGE patterns; this is a powerful approach to characterize the mutation or to localize a small region of DNA in the case of rarer mutations. Am. J. Hematol. 61:120–125, 1999.

Key words: β; thalassemia; mutation; PCR; DGGE; DNA sequencing; India

INTRODUCTION

β-thalassemia is one of the commonest hereditary anemias in India, the prevalence in heterozygotes ranging from 3–15% in different communities [1,2]. At the molecular level, this disorder is extremely heterogenous as more than 180 mutations causing β-thalassemia have been characterized worldwide [3]. So far, 28 mutations have been reported in different ethnic groups originating from the Indian subcontinent [4–11]. In such a situation, one requires a versatile procedure which can identify all known as well as unknown mutations causing β-thalassemia.

Denaturing gradient gel electrophoresis (DGGE) [12] seems to be a very powerful technique where one can identify all nucleic acid alterations or more importantly localize a small fragment of DNA (about 200–400 bp long) containing these alterations.

Here, we report our experience with DGGE to characterize β-thalassemia mutations in the heterogenous Indian population.

MATERIALS AND METHODS

Subjects

356 unrelated β-thalassemia heterozygotes and 55 β-thalassemia homozygotes from various regions in In-

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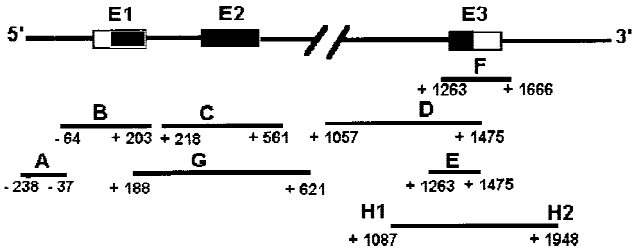

Fig. 1. Schematic diagram of the β-globin gene showing the positions of DGGE fragments (A to G) and the region covering the 619-bp deletion (H).

dia were analysed. Five cases of HbE-thalassemia, three cases of HbD-thalassemia, one case of HbS-thalassemia, three cases of HbE trait, three cases of HbS trait, and two cases of HbD trait were also studied. Ten ml of peripheral blood was collected in EDTA and DNA was prepared from white blood cells by using a standard protocol [13].

Detection of the 619-bp Deletion

This mutation was detected by amplifying fragment H of 861 bps by using a set of specific primers that covered the area of the deletion [7] (Fig. 1). The polymerase-chain-reaction product was visualized after electrophoresis in a 2% Nu-Sieve agarose gel after staining with ethidium bromide. The presence of the mutation was indicated by a smaller fragment of 242 bps.

DGGE

The strategy developed by Ghanem et al. [14] for characterizing Mediterranean thalassemic alleles was followed. The β-globin gene was screened from position -238 at the 5′end to postion +1666 at the 3′ end including the polyadenylation site by amplifying seven overlapping fragments viz. A, B, C, D, E, F, and G (Fig. 1) by using different sets of primers. One of the primers of each pair had a "GC clamp" at the 5′ end to alter the melting profile of the domain [15]. The sequence of the primers was as described earlier [14]. Fragments B, C, and D contain one polymorphic site each (codon 2 C/T, IVS II-16 C/G, IVSII-666 C/T, respectively), whereas fragment G contains three polymorphic sites (IVSII-16 C/G, 74 G/T, and 81 C/T) which define the sequence frameworks (FW 1, 2, 3, 3a) of the β-globin gene.

DGGE was carried out as described by Myers et al. [16]. The 6.5% polyacrylamide gel containing a linear gradient of denaturant (100% denaturant = 7 M urea and 40% formamide) was run at 160 V and 60°C. Fragments A, B, C, and E were analysed by using a 30–80% gradient while fragments G, D, and F were analysed by using a 10–60% gradient. The electrophoresis was run for 5 hr for all the fragments except for fragment F, which required a 4-hr run.

TABLE I. Spectrum of Mutations Identified in β-Thalassemia Heterozygotes by DGGE

Mutations	No. of individuals (%)	Fragment showing the mutation	No. of DGGE patterns
IVSI-5 (G → C)	174 (48.88)	B	4
619bp deletion	54 (15.17)	—	—
Codons 8/9 (+G)	29 (8.15)	B	2
Codons 41/42 (−TTCT)	23 (6.46)	C	2
IVSI-1 (G → T)	23 (6.46)	B	2
Codon 15 (TGG → TAG)	12 (3.37)	B	2
Codon 30 (G → A)	9 (2.53)	B	2
Cap site +1 (A → C)	9 (2.53)	B	2
Codon 30 (G → C)	7 (1.97)	B	2
Codon 121 (G → T)	2 (0.56)	E	1
IVSI-1 (G → A)	2 (0.56)	B	2
Codon 5 (−CT)	1 (0.28)	B	1
Codon 10 (GCC → GCA)	1 (0.28)	B	1
−28 (A → G)	1 (0.28)	B	1
−88 (C → T)	1 (0.28)	A	1
Normal pattern in all fragments	3 (0.84)	—	—
Uncharacterized	2 (0.56)	B	2
	2 (0.56)	C	2
	1 (0.28)	D	1
Total	**356**		**30**

DNA Sequencing

Double-stranded DNA sequencing was carried out by the dideoxynucleotide chain termination method [17] by using the sequenase version 2.0 kit (USB, Cleveland, OH). B_{11} and B_2 were used as sequencing primers for fragment B; C_{11} and C_2 were the sequencing primers for fragment C, and E_1 and D_{22} were the sequencing primers for fragment E. The sequences of B_{11}, C_{11} and D_{22} were the same as those of B_1, C_1, and D_2 [14], but without the "GC clamp."

Preparation of a Catalogue

The mutation corresponding to each anomalous DGGE pattern was characterized by sequencing of the particular fragment showing the atypical pattern. In this way, a catalogue of different DGGE patterns corresponding to various mutations was prepared. Subsequently, a mutation was identified by comparing the DGGE pattern with that from the catalogue.

RESULTS

30 anomalous DGGE patterns were detected after scanning the entire β-globin gene in 356 heterozygotes (Table1). Twenty-five of these patterns were sequenced. They corresponded to 15 different β-thalassemia mutations. The majority of these mutations were detected in fragment B, which covers the promoter region, exon-1, and part of IVS-I. Three samples showed normal DGGE patterns in all fragments, suggesting that the causative

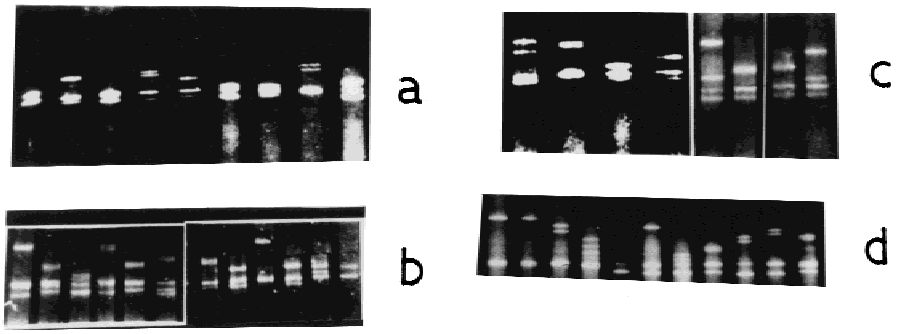

Fig. 2. (a) DGGE patterns of β-thalassemia heterozygotes by fragment B analysis. Left to right: 1) IVSI-5 (G → C) (codon 2 T/T); 2) IVSI-5 (G → C) (codon 2 C/T), mutation linked FW 3a; 3) IVSI-5 (G → C) (codon 2 C/T) mutation linked to FW1; 4) IVS I-5 (G → C) (codon 2 C/C) mutation linked to FW1; 5) normal control (codon 2 C/T); 6) codon 30 (G → C) (codon 2 C/C); 7) IVSI-1 (G → T) (codon 2 C/C); 8) IVSI-1 (G → T) (codon 2 C/T); and 9) codons 8/9 (+G) (codon 2 C/C). (b) DGGE patterns by fragment B analysis. Left to right: First gel: 1) codons 8/9 (+G) heterozygote (codon 2 C/T); 2) HbE trait (codon 2 C/T); 3) codon 30 (G → C) heterozygote (codon 2 C/C); 4) HbS trait (codon 2 C/T); 5) normal control (codon 2 C/T); and 6) codon 5 (-CT) heterozygote (codon 2 C/T). Second gel: 1) IVSI-5 (G → C) heterozygote (codon 2 C/T); 2) HbE trait (codon 2 C/T); 3) IVSI-5 (G → C) +HbE, compound heterozygote; 4) normal control (codon 2 C/T); 5) IVSI-1 (G → A) heterozygote (codon 2 T/T); and 6) -28 (A → G) (codon 2 T/T). (c) Fragment B analysis of some β-thalassemia heterozygotes. Left to right: First gel: 1) cap site + 1 (A → C) (codon 2 C/T); 2) codon 30 (G → A) (codon 2 C/T); 3) codon 30 (G → A) (codon 2 C/C); 4) normal control (codon 2 C/T). Second gel: 1) codon 15 (TGG → TAG) (codon 2 C/T) mutation linked to FW 3a; and 2) normal control (codon 2 C/T). Third gel: 1) normal control (codon 2 C/T); and 2) IVSI-1 (G → A) (codon 2 C/T). (d) DGGE patterns (fragment B) of β-thalassemia major (compound heterozygotes), β-thalassemia heterozygotes and abnormal hemoglobin cases. Left to right: 1) HbE+ IVSI-5 (G → C); 2) IVSI-5 (GC) + codon 15 (TGG → TAG); 3) codon 15 (TGG → TAG) (codon 2 C/T), mutation linked to FW1; 4) IVSI-5 (G → C)+ -28 (A → G); 5) normal control (codon 2 C/C); 6) IVSI-1-5(G → C) + codon 30 (G → C); 7) codon 30 (G → C) (codon 2 C/C); 8) Normal control (codon 2 C/T); 9) HbS+ Codons 8/9 (+G);10) HbS trait (codon 2 C/T); and 11) IVSI-5 (G → C) (codon 2 C/T).

mutation was probably lying outside the β-globin gene, possibly in the locus control region (LCR). Some of the anomalous DGGE patterns in fragment B are shown in Figs. 2 and 3. It is interesting to note that in Fig. 2a, the first four patterns (lanes1–4) represent the same mutation viz. IVSI-5(G → C). Such a situation arises, when the mutation lies in a fragment that also contains a silent polymorphism (e.g., T or C in codon 2 in fragment B). Here, the DGGE pattern is the result of interaction between the polymorphism and the mutation. The pattern changes according to the framework of the wild-type chromosome present. The first two patterns of the IVSI-5 (G → C) mutation result from the presence of a T and a C, respectively, at codon 2 on the wild-type chromosome. This mutation is generally linked to FW3a, which has a T at codon 2. Therefore, in the above-mentioned patterns, the mutant allele has a T at codon 2. In lanes 3 and 4, the wild-type chromosome has a C and a T, respectively, at codon 2 but in these two samples the mutation is linked to FW1 (this was confirmed by family studies). Therefore, the mutant allele has a C at codon 2. Figs. 2b and c depict some more DGGE patterns of fragment B corresponding to different mutations . Lane 6 of the second gel in Fig. 2b represents the DGGE pattern for the rare Chinese mutation -28 (A → G), described for the first time in Indians. It is therefore apparent that several mutations are represented by more than one DGGE pattern. Common abnormal hemoglobins like HbE or HbS can also be detected by analysing this fragment.

Among the 55 β-thalassemia major cases, 25 cases were homozygous for the same mutation. They were represented by a single band (mutant homoduplex) with a mobility slightly different from the normal homoduplex band. Sixteen cases were double heterozygous where one of the mutations was the 619-bp deletion. Twelve cases were double heterozygotes with both mutations being present in the same fragment, whereas in the remaining two cases, the two mutations were located in different fragments. Some of these distinct fragment B patterns are demonstrated in Fig. 2d. Lanes 1 and 2 show identical DGGE patterns in different individuals who were double heterozygotes of the IVSI-5 (G → C) mutation in combination with the codon 15 (TGG → TAG) and HbE [codon 26 (GAG → AAG)] mutations, respectively. This was confirmed by family studies and DNA sequencing. Lane 3 reveals the DGGE pattern in a heterozygote for the codon 15 (TGG → TAG) mutation. This pattern is again different from the codon 15 (TGG → TAG) pattern shown in the second gel of Fig. 2c. In both these cases, although the codon 2 polymorphism is the same, the difference in patterns is due to linkage of this mutation to different frameworks. The normal control in all the figures shows the presence of both, homoduplexes and heteroduplexes due to the polymorphism at codon 2.

Figure 3a illustrates analysis of fragment G showing 10 possible combinations of four frameworks viz. 1, 2, 3, and 3a.

Two patterns of the codons 41/42(-TTCT) mutation which is located in fragment C are demonstrated in Fig. 3b. These two patterns are seen due to difference in the framework of the β^A chromosome. This figure also demonstrates two patterns of an uncharacterized mutation located in fragment C, as well as the detection of HbD. DGGE patterns of two rare β-thalassemia mutations viz:

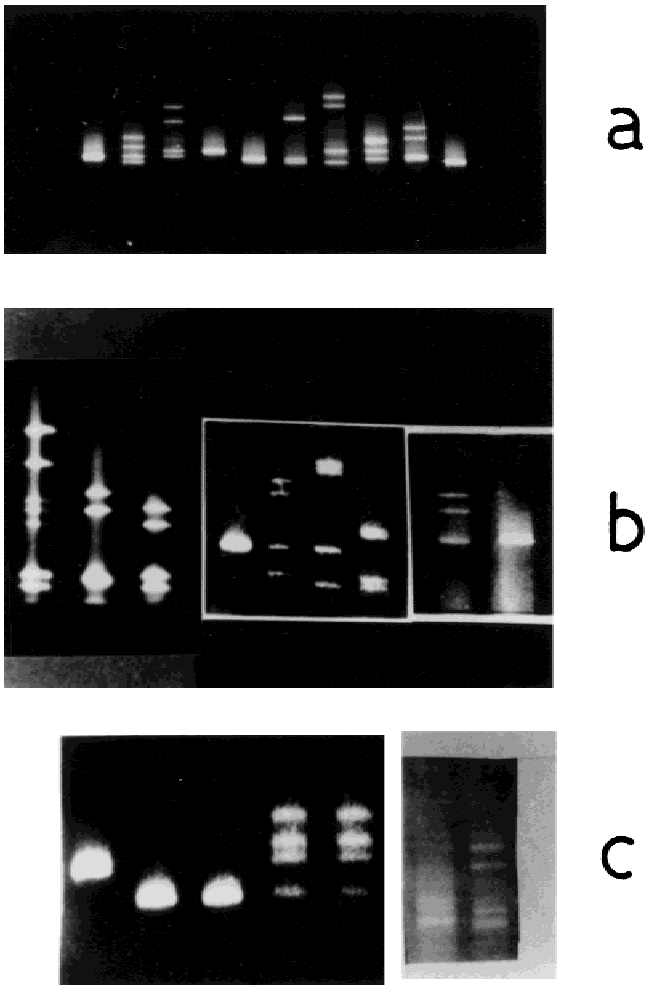

Fig. 3. (a) Fragment G analysis showing different combinations of frameworks. Left to right: 1) FW 2/2; 2) FW 1/2; 3) FW 2/3; 4) FW 3/3; 5) FW 3a/3a; 6) FW 1/3a; 7) FW 1/3; 8) FW 3/3a; 9) FW 2/3a; and 10) FW 1/1. (b) DGGE analysis of fragment C and fragment E. Left to right: First gel: fragment C analysis: 1) codons 41/42 (-TTCT) heterozygote (IVS II-16 C/C); 2) codons 41/42 (-TTCT) heterozygote (IVS II-16 C/G); and 3) normal control (IVS II-16 C/G). Second gel: fragment C analysis of a family where the mutation is not yet characterized: 1) β-thal homozygote; 2) β-thal heterozygote (father); 3) β-thal heterozygote (mother); 4) normal control (IVS II-16 C/G). Third gel: fragment E analysis: 1) HbD trait; 2) normal control. (c) DGGE pattern of two rare β-thalassemia mutations. Left to right: First gel: fragment E analysis 1) normal control; 2) and 3) codon 121 (G → T) homozygotes; 4) and 5) codon 121(G → T) heterozygotes. Second gel - fragment B analysis: 1) IVS 1 - 5 (G → C) (codon 2 T/T) 2) codon 10 (GCC → GCA) (codon 2 C/T).

codon 121 (G → T) and codon 10 (GCC → GCA) are shown in Fig. 3c.

Results of double heterozygotes of β-thalassemia with abnormal hemoglobins showed that all the 5 cases of HbE had the IVSI-5 (G → C) mutation, HbS was present with codons 8/9(+G), whereas the 3 HbD thalassemia cases showed the 619-bp deletion, IVSI-5(G → C) and codons 41/42(-TTCT) mutations, respectively.

TABLE II. Framework Linkage Analysis of β-thalassemia Mutations and Abnormal Hemoglobins

Mutation	Framework 1	2	3	3a	No. of chromosomes
IVSI-5 (G → C)	8	2	—	102	112
619 bp deletion	1	—	—	29	30
Codons 8/9 (+G)	17	—	—	—	17
Codon 30 (G → A)	6	—	—	—	6
IVSI-1 (G → T)	12	—	—	—	12
Codon 15 (TGG → TAG)	2	—	—	5	7
IVSI-1 (G → A)	—	—	2	—	2
Cap site + 1 (A → C)	—	1	3	—	4
Codon 30 (G → C)	3	—	—	—	3
Codon 26 (GAG → AAG) HbE	—	3	—	—	3
Codon 6 (GAG → GTG) HbS	—	3	—	—	3
−28 (A → G)	—	—	—	1	1
Codon 10 (GCC → GCA)	—	1	—	—	1
Codon 121 (GAA → CAA) HbD	3	—	—	—	3
Codon 121 (G → T)	—	2	—	—	2

Table 2 reveals the linkage of various β-thalassemia mutations to different frameworks which was confirmed by family studies. Four mutations showed linkage with more than one framework. Linkage of IVSI-5(G → C) to FW2, 619-bp deletion to FW1 and cap site +1(A → C) to FW2 is being reported for the first time.

DISCUSSION

β-thalassemia is the most common monogenic disease in the Indian sub-continent. An intricate caste system and the practice of endogamy is responsible for genetic diversity in the population. Although β-thalassemia has been reported in 23 caste groups with varying frequencies [2], the profile of mutations amongst them has not been fully elucidated. It was therefore necessary to adopt a technique which can identify all the known molecular lesions, as well as be useful in harvesting new mutations in an extremely diverse population.

Amongst the various protocols available today, DGGE has the distinct advantage of characterizing almost all point mutations, small insertions and deletions as well as neutral polymorphisms of the β-globin gene. It has been applied successfully for characterizing β-thalassemia mutations in the Chinese population [18], in Northern Europe [12], in Sardinia [19], and in the Mediterranean population [20]. So far, its potential for characterization of different mutations in the Indian population has not been fully explored. DGGE has an added advantage because fragment G analysis is useful to determine the framework of the β-globin gene and the linkage of the mutation to different frameworks in informative families.

We recently applied the DGGE procedure to offer first trimester prenatal diagnosis of β-thalassemia in a few families [21].

In the present study, we are reporting the DGGE patterns for the codon 10 (GCC → GCA) and codon 121(G → T) mutations for the first time. Further, in five cases, the mutations have yet to be identified; nevertheless, we were able to localize small regions of the β-globin gene where these mutations lie. This will avoid extensive sequencing of the entire gene, an advantage DGGE has over conventional approaches like ARMS or Dot Blot Hybridization.

Codon 121 (G → T), a rare mutation of exon 3, initially reported in French, Polish and Japanese individuals [22–24] was detected in both the partners of a nonconsanguinous marriage. They had a child suffering from severe β-thalassemia major. This mutation was associated with FW2 like the Japanese case [24]. The mutation was confirmed by sequencing as well as by *Eco*RI digestion as the restriction site for this enzyme is abolished by the mutation (unpublished data).

The 619-bp deletion was earlier reported to be linked to a single haplotype associated with FW3 [25,26] and this was confined to the Sindhi community. On the basis of this, a single origin of this mutation was suggested. In the present series, we observed association of this mutation with FW 1 also in a Sindhi individual. This was confirmed by family studies. This raises the possibility of a new independent origin of the same mutation.

Before applying DGGE as a diagnostic method, certain points must be considered. The mobility of homoduplexes and heteroduplexes of amplified DNA in DGGE depends on its nucleic acid sequence. When the mutation in a β-thalassemia heterozygote is present in a fragment which also contains neutral polymorphisms (e.g., fragments B, C, or D), the mobility is determined by the combined effect of the polymorphism and the mutation as explained earlier. The situation becomes more complicated when the same β-thalassemia mutation is linked to more than one framework [26,27]. All these factors are responsible for getting multiple DGGE patterns corresponding to the same mutation. In the present series, the IVS I-5 (G → C) mutation was found to be linked to three different frameworks and is represented by four distinct DGGE patterns, whereas most of the other mutations are represented by two DGGE patterns. Therefore, preparation of a comprehensive catalogue of DGGE patterns is an important prerequisite.

A word of caution while analysing β-thalassemia major cases. It is very difficult to differentiate between DNA samples which are homozygous for the same mutation and those homozygous for the polymorphism (e.g., C/C or T/T in fragment B). This can be resolved by artificial creation of heterozygous patterns of β-thalassemia mutations present in thalassemia major cases [21]. Double heterozygous cases where both the mutations are in the same fragment, exhibit completely different patterns from those of the individual mutations. In rare cases, such as those we have encountered in the present study, if different combinations of mutations finally result in the same substitutions, identical DGGE patterns are obtained. Family studies are very important in such cases.

Thus considerable initial work up is required before one can scan the entire β-globin gene in two electrophoretic runs for detection of known as well as unknown mutations. Detection of novel and rare mutations like codon 10 (GCC → GCA), codon 121(G → T), and -28(A → G) shows the increasing repertoire of molecular defects causing β-thalassemia among Indians which can be unearthed by using a scanning technique like DGGE.

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REFERENCES

1. Sukumaran PK, Master HR. The disribution of abnormal hemoglobins in the Indian population. In: Sanghvi LD, Balkrishnan V, Bhatia HM, Sukumaran PK, Undevia JV, editors. Human Population Genetics in India, Volume 1. Ind Soc Hum Genet 1974. p 91.
2. ICMR Task Force Study. Collaborative study on thalassemia Indian Council of Medical Research, New Delhi, 1993.
3. Baysal E, Carver MFH. The β-and δ-thalassemia repository Hemoglobin 1994;19:213.
4. Kazazian HH Jr, Orkin SH, Antonarakis SE, Sexton JP, Boehm CD, Goff SC, Waber PG. Molecular characterization of seven β-thalassemia mutations in Asian Indians. EMBO J 1984;3:593.
5. Thein SL, Hesketh C, Wallace RB, Weatherall DJ. The molecular basis of thalassemia major and thalassemia intermedia in Asian Indians: application to prenatal diagnosis. Brit J Hematol 1988;70:225.
6. Varawalla NY, Old JM, Sarkar R, Venkatesan R, Weatherall DJ. The spectrum of β-thalassemia mutations on the Indian sub-continent: the basis for prenatal diagnosis. Brit J Hematol 1991a;78:242.
7. Varawalla NY, Old JM, Weatherall DJ. Rare β-thalassemia mutations in Asian Indians. Brit J Hematol 1991b;79:640.
8. Gupta RB, Tiwary RS, Pande PL, Kutlar F, Oner C, Oner R, Huisman THJ: Hemoglobinopathies among the Gond tribal groups of Central India: interaction of α and β-thalassemia with β-chain variants. Hemoglobin 1991;15:441.
9. Baysal E, Sharma S, Wong SC, Jogessar VB, Huisman THJ. Distribution of β-thalassemia mutations in three Asian Indian populations with distinct geographical locations.Hemoglobin 1994;18:201.
10. Garewal G, Fearon CW, Warren TC, Marwaha N, Marwaha RK, Mahadik C,Kazazian HH Jr. The molecular basis of β-thalassemia in Punjabi and Maharashtrian Indians includes a multilocus aetiology involving triplicated α-globin loci. Brit J Haematol 1994;86:372.
11. Pawar AR, Colah RB , Mohanty D. A novel β^+-thalassemia mutation (codon10 GCC → GCA) and a rare transcriptional mutation (-28 A → G) in Indians. Blood 1997;89:3888.

12. Losekoot M, Fodde R, Harteveld CL, Van Heeren H, Giordano PC, Bernini LF. Denaturing gradient gel electrophoresis and direct sequencing of PCR amplified DNA - a rapid and reliable diagnostic approach to β-thalassemia. Brit J Hematol 1990;76:269.
13. Elles R, Old JM. Laboratory procedures for DNA analysis. WHO training course in standard techniques and advanced methodologies for the control of hereditary anemias. Crete, Herakleion, pp. 5, 1987.
14. Ghanem N, Girodon E, Vidaud M, Martin J, Fanen P, Plassa F, Goossens M. A comprehensive scanning method for rapid detection of β-globin mutations and polymorphisms. Hum Mutation 1992;1:229.
15. Sheffield V, Cox DR, Lerman LS, Myers RM. Attachment of a GC clamp to genomic DNA fragments by the polymerase chain reaction results in improved detection of single base changes. Proc Natl Acad Sci USA 1989;86:232.
16. Myers RM, Maniatis T, Lerman LS. Detection and localization of single base changes by denaturing gradient gel electrophoresis. Methods Enzymol 1987;155:501.
17. Sanger F, Nicklen S, Coulson AR. DNA sequencing with chain terminating inhibitors.Proc Natl Acad Sci USA 1977;74:5463.
18. Cai SP, Kan YW. Identification of the multiple β-thalassemia mutations by Denaturing Gradient Gel Electrophoresis.J Clin Invest 1990; 85:550.
19. Rosatelli MC, Dozy A, Faa V, Meloni A, Sardu R, Saba L, Kan YW, Cao A. Molecular characterization of β-thalassemia in the Sardinian population. Am J Hum Genet 1992;50:422.
20. Dozy AM, Kan YW. Characterization of β-thalassemia mutations by denaturing gradient gel electrophoresis: patterns in the Mediterranean mutations. Clin Genet 1994;45:221.
21. Gorakshakar AC, Lulla CP , Nadkarni AH, Pawar AR, Desai SN, Colah RB, Mohanty D. Prenatal diagnosis of β-thalassemia among Indians using denaturing gradient gel electrophoresis Hemoglobin 1997;21:421.
22. Kazazian HH Jr, Orkin SH, Boehm CD, Goff SC, Wong C, Dowling CE, Newburger PE, Knowlton RG, Brown V, Donis-Keller H. Characterization of a spontaneous mutation to a β-thalassemia allele. Am J Hum Genet 1986;38:860.
23. Fei YJ, Stoming TA, Kutlar A, Huisman THJ, Stamatoyannopoulos G: one form of inclusion body β-thalassemia is due to a GAA →TAA mutation at codon 121 of the β-chain. Blood 1989;73:1075.
24. Yamamoto Ku, Yamamoto Ki, Hattori Y, Yamashiro Y, Hoshitani M, Morishita M, Ohba Y, Katahira H, Karasawa M, Omine M, Narukiyo T, Hirabayashi K, Miyawaki S. Two β-thalassemia mutations in Japan: Codon 121 (GAA → TAA) and IVS 1-130 (G → C). Hemoglobin 1992;16:295.
25. Thein SL, Old JM , Wainscoat JS, Petrou M, Modell B, Weatherall DJ. Population and genetic studies suggest a single origin for the Indian deletion β^0 thalassemia. Brit J Hematol 1984;57:271.
26. Varawalla NY, Fitches AC, Old JM. Analysis of β-globin gene haplotypes in Asian Indians: origin and spread of β-thalassemia on the Indian sub-continent. Hum Genet 1992;90:443.
27. Orkin SH, Kazazian HH Jr, Antonarakis SE, Goff SC, Boehm CD, Sexton JP, Waber PG, Giardina PJV. Linkage of β-thalassemia mutations and β-globin gene polymorphisms with DNA polymorphisms in human β-globin gene cluster. Nature (London) 1982;296:627.